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# Primary breast diffuse large B-cell lymphoma shows a non-germinal center B-cell phenotype

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**Primary breast diffuse large B-cell lymphoma has a poor prognosis relative to other extranodal diffuse large B-cell lymphoma. Recently, diffuse large B-cell lymphoma has been subclassified as germinal center B-cell-like and nongerminal center B-cell types using tissue microarrays. The 5-year overall survival rate of the germinal center B-cell group is better than that of the nongerminal center B-cell group. To elucidate the reason for which primary breast diffuse large B-cell lymphoma has a poor clinical outcome, we investigated 15 patients with primary breast diffuse large B-cell lymphoma (stage IE; 13 cases, stage IIE; two cases) by immunohistochemistry using various markers including CD10, Bcl-6, MUM1 and MIB-1 and by molecular analysis of the immunoglobulin heavy chain gene variable region. Immunohistochemistry showed 0/15 (positive cases/examined cases) for CD10, 5/15 for Bcl-6, 15/15 for MUM1, 10/15 for Bcl-2, 2/15 for CD5 and 4/15 for CD40. The expression pattern of CD10(−) MUM1(+) in primary breast diffuse large B-cell lymphoma corresponded to the nongerminal center B-cell group. Moreover, the MIB-1 index was distributed from 60 to 95% with a mean of 79%, indicating a high proliferation of the lymphoma cells. The immunoglobulin heavy chain gene variable region of primary breast diffuse large B-cell lymphoma had a mutation frequency of 1–10% (seven cases) and 0–1 additional mutations in ongoing mutation analysis (five cases). Primary breast diffuse large B-cell lymphoma had characteristics of the nongerminal center B-cell group. In conclusion, primary breast diffuse large B-cell lymphoma has a nongerminal center B-cell phenotype and has a high MIB-1 index. These features might therefore be associated with poor prognosis.**
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**Keywords:** lymphoma; breast; diffuse large B-cell lymphoma; nongerminal center B cell; immunoglobulin heavy chain gene variable region; ongoing mutation; MUM1

Primary breast lymphoma is a very uncommon condition that accounts for 0.05–0.53% of all malignant diseases of the breast and 2.2% of extranodal malignant lymphomas.[1–12] Clinicopathologic characteristics of primary breast lymphoma have been well investigated to date. Almost all primary breast lymphoma has a B-cell phenotype, while primary breast lymphoma with T-cell phenotype is extremely rare. In all, 46–71% of primary breast lymphoma are diffuse large B-cell lymphomas (DLBCL).[2,5–9,13] The age distribution of primary breast lymphoma patients is bimodal; younger patients with a peak of 30–35 years frequently constitute DLBCL and a small number of Burkitt lymphomas with bilateral tumors, whereas older patients with a peak of 55–60 years constitute DLBCL and/or marginal zone B-cell lymphomas of mucosa-associated lymphoid tissue (MALT-type lymphoma) with unilateral tumors.[8,9,11] Primary breast lymphoma is reported to exhibit a poor prognosis among extranodal B-cell lymphomas. The overall survival rate of primary breast lymphoma with a B-cell phenotype is 43% at 5 years. This is worse than those reported in the thyroid: 79% 5-year disease-specific survival[14] and Waldeyer's ring: 70% 5-year overall survival,[15] but is better than central nervous system (CNS) lymphomas: 37% 2 year overall survival.[16] Moreover, the 5-year overall survival of the gastrointestinal high-grade B-cell lymphoma is 63%,[17] on the other hand, the median survival of primary breast DLBCL is reported to be 36 months.[18] The reasons for which primary breast lymphoma, especially DLBCL is worse compared to other extranodal malignant lymphoma is unknown.

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It has been recently shown that DLBCL can be divided into three prognostically important subgroups: germinal center B-cell-like DLBCL, activated B-cell-like DLBCL , and type 3 by gene expression profiles using a cDNA microarray.[19,20] Germinal center B-cell-like DLBCL has a better clinical outcome than activated B-cell and type 3. Hans *et al*[21] subsequently reported that the immunohistochemical expression pattern of CD10, Bcl-6 and MUM1 can be used to categorize DLBCL into and nongerminal center B-cell type, including activated B-cell and type 3, with an outcome similar to that predicted by cDNA microarray analysis.

In this article, we therefore investigate 15 patients with primary breast DLBCL in stages I and II, with immunohistochemistry using various markers including germinal center B-cell markers, nongerminal center B-cell type markers and MIB-1, and molecular analysis of the immunoglobulin heavy chain gene variable region (IgH-V gene) to elucidate the biological characteristics of primary breast lymphoma.

## Patients and methods

### Patients

In all, 15 patients with primary breast lymphoma at stage IE (13 cases) or at stage IIE (two cases) were retrieved from the lymphoma files of Department of Pathology at Fukushima Medical University. Because breast lymphoma with generalized disease in the III and IV clinical stages is considered to be primary or secondary lymphoma, these patients were excluded from this study. Patients with bilateral lesions were also excluded in order to carry out immunohistochemical and biological analysis for primary lesions.

All patients underwent surgery for either tumor excision (four cases) or mastectomy (11 cases). All patients fulfilled the criteria for primary breast lymphoma proposed by Wiseman and Liao:[10] adequate pathological specimen, close association of breast tissue and lymphomatous infiltrate, and no other lymphomatous focus at the time of diagnosis except for the presence of ipsilateral axillary nodes provided that nodal involvement occurred concomitantly with the primary breast lesion. Patients were assigned to either IE or IIE stages at the time of diagnosis, according to the Ann Arbor staging system applied to primary extranodal lymphomas.[22]

### Routine Light Microscopy

Resected tissue was fixed in formalin and embedded in paraffin. A portion of the sample of four cases was frozen. Sections were stained with hematoxylin and eosin, and primary breast DLBCL was diagnosed according to the World Health Organization Classification of Tumors of Haematopoietic and Lymphoid Tissue.[23]

### Immunohistochemistry

Immunoperoxidase staining was performed using an avidin–biotin-peroxidase complex technique on both paraffin-embedded sections and frozen sections.[24] Deparaffinized and dehydrated tissue sections were pretreated by microwaves and stained with murine antibodies (Table 1). The MIB-1 index was evaluated for proliferative activity of lymphoma cells. Well-stained areas in MIB-1 and CD20 double stainings were selected. Doubly positive large cells (MIB-1 plus CD20) and CD20-positive large cells were counted under high-power fields ( $\times 400$). At least 350 CD20-positive cells were counted. The mean percentage under three high-power fields was considered to be the MIB-1 index.

### Molecular Investigation

DNA was extracted from paraffin-embedded or frozen tissues using a DNeasy Tissue Kit (Qiagen Co., Valencia, CA, USA). The variable (CDR1, FW2, CDR2 and FW3) and the VDJ regions (CDR3) of the

**Table 1** Antibodies used for immunohistochemical staining—paraffin sections

| *Antibody* | *Clone* | *Source* |
|---|---|---|
| CD3 | CD3 | DA |
| CD5 | 4C7 | NC |
| CD10 | 56C6 | NC |
| CD20 | FB-1 | Our laboratory |
| CD21 | 1F8 | DA |
| CD23 | 1B12 | ST |
| CD27 | 1A4 | NC |
| CD30 | Ber-H2 | DA |
| CD40 | 11E9 | NC |
| CD56 | 123C3 | ZL |
| CD138 | B-B4 | DS |
| Bcl-2 | 124 | DA |
| Bcl-6 | N3 | SC |
| IRF4/MUM1 | M-17 | SC |
| AE1/3 | AE1 and AE3 | DA |
| MIB-1 | MIB-1 | IT |
| Granzyme B | GrB-7 | KM |
| LMP1 | CS1-CS4 | DA |
| Pax5 | N-19 | SC |
| p21 | EA10 | OR |
| p27 | p27 | BD |
| p53 | DO7 | DA |
| TdT | TdT | DA |
| TIA-1 | 2G9 | IT |

BD: Becton Dickinson, San Jose, CA, USA; DA: Dakocytometer, Copenhagen, Denmark; DS: Dainipponseiyaku, Osaka, Japan; EB: Epstein–Barr virus IT: Immunotech, Marseille, France; KM: Kamiya Medical Company, Seattle, WA, USA; NC: Novocastra laboratories LtD., Newcastle upon tyne, UK; OR: Oncogene Research Products, San Diego, CA, USA; ST: Serotec LTD., Oxford, UK; SC: Santa Cruz Biotechnology Inc., San Diego, USA; ZL: Zymed laboratories Inc., San Francisco, USA.

immunoglobulin heavy chain (IgH) gene were amplified by seminested polymerase chain reaction (PCR) as previously described.[25,26] The primers used in this study were as follows: 5′-AGGTGCAGCTG[C/G][A/G/T]G[C/G]AGTC[A/G/T]GG-3′(FR1C) and 5′-TGG[A/G] TCCG[C/A] CAG [G/C] C [T/C][T/C] C [A/C/G/T] GG-3′(FR2A), as an upstream consensus V region primer; 5′-TGAGGAGACGGTGACC-3′, as a consensus J region primer (LJH); and 5′-GTGACCAG GGT[A/C/G/T]CCTTGGCCCC AG-3′, as a consensus J region primer (VLJH).[25] The nucleotide sequences between CDR1 and FW3 or CDR2 and FW3 were analyzed using HITACHI SQ-5500 (Tokyo, Japan) and compared with the germline sequences recorded in the gene bank database. Somatic mutations of the IgH gene variable region (IgH-V gene) were analyzed using the Ig blast site (http://www.ncbi.nlm.nih.gov/igblast/).

The presence or absence of ongoing mutations was determined according to a previously described method.[27] Briefly, PCR products were ligated into the PCR$^{R}$ 2.1 vector and transformed into TOP10F′ cells according to the manufacturer's instructions (original TA cloning kit; Invitrogen, Carlsbad, CA, USA). A colony direct PCR assay was used to determine whether colonies included the correct PCR product. A total of 10 or more white colonies were selected and placed into 50 $\mu$l of Insert Check ready (TOYOBO, Osaka, Japan). PCR amplification consisted of 30 cycles of 95°C for 20 s, 60°C for 5 s and 72°C for 30 s. Then, 10 samples including correct PCR products confirmed by check-electrophoresis for each case were sequenced by the same method.

## Results

### Clinical Features

The clinical features and outcomes of patients are summarized in Table 2. Age at diagnosis ranged from 45 to 81 years (median, 68). Four patients with tumors under 5 cm did not relapse. Six (all belonged to stage IE) of 11 patients with tumors larger than 5 cm were relapsed. Of the six patients who relapsed, four died of lymphoma. Both patients with stage IIE survived and did not relapse. There was no relationship among tumor size, LDH ratio, IPI and staging.

### Histological and Immunohistochemical Findings

Histological and immunohistochemical findings in paraffin sections are shown in Table 3. The lymphoma constituted a poorly circumscribed mass, infiltrated the breast lobules and surrounded breast ducts. All patients showed a diffuse infiltration of closely packed large-sized lymphoma cells, and a diagnosis of DLBCL was made (Figure 1). A

**Table 2** Summary of the clinical features of primary mammary diffuse large B-cell lymphoma

| *No.* | *Age* | *Size (cm)*[a] | *Side* | *LDH ratio* | *IPI*[b] | *Stage* | *Operation* | *Chemotherapy* | *Relapse* | *Outcome* | *Follow-up (month)* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 77 | 7.0 × 6.0 | Rt. | 1.92 | 2 | IIE | MRM | CHOP | | Alive | 7 |
| 2 | 63 | 5.6 × 4.8 | Lt. | | 1/2 | IE | Excision | MACOP-B | Bladder, abdominal tumor[c]; 43 mo | AWD | 46 |
| 3 | 60 | 2.1 × 1.8 | Lt. | | 1/2 | IE | MRM | THP-COP | | Alive | 35 |
| 4 | 45 | 4.0 × 3.0 | Rt. | 1.10 | 1 | IE | Excision | CHOP | | Alive | 5 |
| 5 | 68 | 6.5 × 3.5 | Rt. | 1.89 | 2 | IE | RM | VEPA | | Alive | 171 |
| 6 | 63 | 9.8 × 8.5 | Lt. | 0.94 | 1 | IE | MRM | None | Subcutaneous tumor (left chest wall); 1 mo | DOD | 5 |
| 7 | 71 | 2.6 × 2.4 | Lt. | | 1/2 | IE | MRM | THP-COP | | Alive | 63 |
| 8 | 73 | 5.0 × 4.0 | Lt. | 1.21 | 2 | IE | RM | CHOP, CPA+VC+VDS | Local, bone marrow, spleen; 10 mo | DOD | 17 |
| 9 | 77 | 2.8 × 2.8 | Lt. | 1.92 | 2 | IE | Excision | THP-COP+RT | | Alive | 8 |
| 10 | 69 | 5.2 × 5.1 | Lt. | 1.16 | 2 | IE | MRM | None | | Alive | 3 |
| 11 | 81 | 6.8 × 4.9 | Lt. | 1.03 | 2 | IE | MRM | Half-CHOP without ADM | Mandible lymph node; 13 mo | DOD | 16 |
| 12 | 66 | 6.5 × 5.8 | Rt. | 0.80 | 1 | IE | MRM | CHOP | Opposite breast; 81 mo | AWD | 81 |
| 13 | 76 | 7.0 × 6.0 | Lt. | 0.75 | 1 | IIE | MRM | low-dose CHOP | | Alive | 12 |
| 14 | 60 | 9.5 × 6.5 | Lt. | 1.02 | 1 | IE | MRM | CHOP | | Alive | 4 |
| 15 | 53 | 5.5 × 5.5 | Lt. | | 0/1 | IE | Excision | VEPA | Opposite breast; 38 mo | DOD | 94 |

Alive: alive without disease; AWD: alive with disease; CHOP: cyclophosphamide, adriamycin, vincristine, prednisolone; DOD: dead on disease; IPI: International Prognostic Index; LDH ratio: measured value/normal value; MRM: modified radical mastectomy; MACOP-B: methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisolone, bleomycin; RT: radiation therapy; RM: radical mastectomy; THP-COP: cyclophosphamide, THP-doxorubicin (pirarubicin), vincristine, prednisolone; VC: vincristine; VDS: vindesine; VEPA: vinblastine, etoposide, prednisone, doxorubicin; mo: months.

[a]Tumor size was decided by palpation.

[b]a/b means that IPI was a or b because the LDH ratio of the patient was unknown.

[c]relapse lesions in bladder and abdomen were DLBCL.

**Table 3** Histological and immunohistochemical features in paraffin sections and *in situ* hybridization (ISH)

| *No.* | *Histological subtype* | *S-S* | *LEL* | *MIB-1* | *CD3* | *CD5* | *CD10* | *CD20* | *CD21* | *CD23* | *CD27* | *CD30* | *CD40* | *CD56* | *CD138* | *Bcl2* | *Bcl6* | *MUM1* | *Gra.B* | *Cyclin D1* | *LMP1* | *p21* | *p27* | *p53* | *TdT* | *TIA-1* | *EBER1 ISH* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | DLBCL with MALT | − | + | 76.7% | − | − | − | + | − | − | − | − | + | − | − | + | − | + | − | − | − | − | + | − | − | − | − |
| 2 | DLBCL with MALT | − | + | 75.2% | − | − | − | + | − | − | − | − | − | − | − | − | + | − | − | − | − | − | + | − | − | − | − |
| 3 | DLBCL | − | + | 83.6% | − | +/− | − | + | − | − | − | − | + | + | − | + | − | + | − | − | − | − | + | − | − | − | − |
| 4 | DLBCL | − | + | 78.5% | − | − | − | + | − | − | − | + | + | − | − | + | + | + | − | − | − | − | − | − | − | − | − |
| 5 | DLBCL | − | + | 72.8% | − | +/− | − | + | − | − | − | − | − | − | − | + | + | + | − | − | − | − | − | − | − | − | NT |
| 6 | DLBCL | − | + | 76.8% | − | − | − | + | − | − | − | − | − | − | − | − | − | + | − | − | − | − | + | − | − | − | − |
| 7 | DLBCL | + | − | 79.9% | − | − | − | + | − | − | − | − | − | − | − | − | + | + | − | − | − | − | + | − | − | − | NT |
| 8 | DLBCL | + | − | 95.2% | − | − | − | + | − | − | − | − | − | − | − | + | − | + | − | − | − | − | − | − | − | − | NT |
| 9 | DLBCL | − | − | 88.3% | − | − | − | + | − | − | − | − | − | − | − | − | + | + | − | − | − | − | − | − | − | − | NT |
| 10 | DLBCL | − | − | 59.5% | − | − | − | + | − | − | − | − | − | − | − | + | − | + | − | − | − | − | + | − | − | − | − |
| 11 | DLBCL | − | − | 83.1% | − | − | − | + | − | − | − | − | + | − | − | + | − | + | − | − | − | − | + | − | − | − | − |
| 12 | DLBCL | + | − | 78.1% | − | − | − | + | − | − | − | − | − | − | − | + | + | + | − | − | − | − | − | − | − | − | − |
| 13 | DLBCL | − | − | 83.8% | − | − | − | + | − | − | − | − | − | − | − | + | − | + | − | − | − | − | − | − | − | − | − |
| 14 | DLBCL | + | − | 84.0% | − | − | − | + | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − |
| 15 | DLBCL | + | − | 72.0% | − | − | − | + | − | − | − | − | − | − | − | + | − | + | − | − | − | − | − | − | − | − | NT |

EBER1: Epstein–Barr virus encoded RNA1; Gra.B: Granzyme B; LEL: lymphoepithelial lesion demonstrated in AE1/3; S-S: starry-sky appearance; +: positive (positive cell ≥20%); −: negative +/−: weakly positive (weakly colored); NT: not tested.



**Figure 1** DLBCL without MALT-type lymphoma (HE, case 8). A diffuse proliferation of large centroblasts are seen. Starry-sky macrophages are present (starry sky appearance).

lymphoepithelial lesion was seen in six cases. Two (cases 1 and 2) were DLBCL with MALT-type lymphoma (Figure 2) and four (cases 3–6) were DLBCL without MALT-type lymphoma.

Immunohistochemistry showed CD3;0/15 (positive cases/examined cases), CD5; 2/15, CD10; 0/15, CD20; 15/15, CD21; 0/15, CD23; 0/15 CD27; 0/15, CD30; 1/15, CD40; 4/15; CD56; 0/15, CD138; 0/15, Bcl-2; 10/15, Bcl-6; 5/15, IRF4/MUM1; 15/15, granzyme B; 0/15, LMP-1; 0/15, cyclin D1; 0/15, p21; 0/15, p27; 7/15, p53; 0/15, TdT; 0/15 and TIA-1; 0/15 (Figure 3). The MIB-1 index ranged from 59 to 95% and the mean MIB-1 index was 79% (Figure 4).

### Molecular Findings

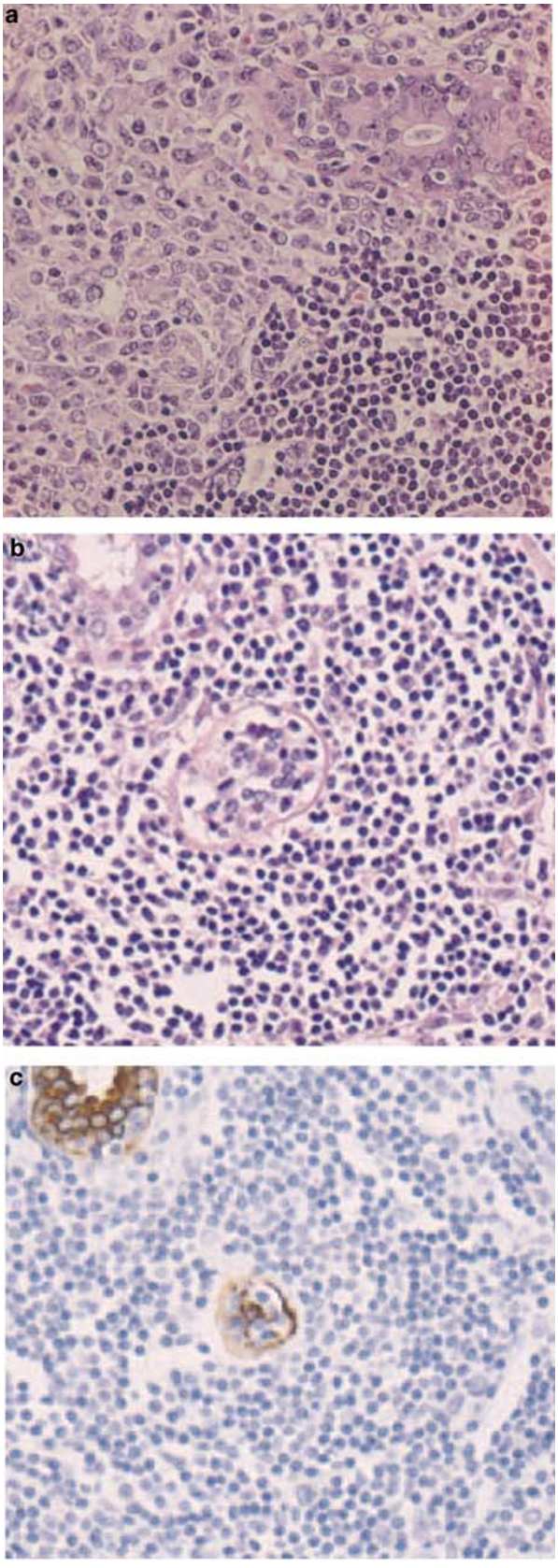
The IgH-V gene was analyzed in seven patients. The results of somatic mutations and ongoing mutations of the IgH-V gene are shown in Table 4. The frequency of mutations ranged from 1 to 10%. The usage of the VH family was VH4 in four cases and VH3 in three cases. Overall, 10 clones were examined for ongoing mutations in five patients. Three cases showed no additional mutations and the other two showed only one additional mutation.

## Discussion

We investigated immunohistochemical and molecular features in 15 patients with primary breast DLBCL to elucidate the biological characteristics of primary breast lymphoma and why primary breast lymphoma has a poor prognosis. For these aims, we selected patients with breast DLBCL in stages IE/IIE, such that this study included patients older than 45 years of age.

All cases immunohistochemically showed CD10(−) and MUM1(+). Bcl-6 was expressed in five of 15 cases. According to the system of Hans

*et al*,[21] DLBCL cases of CD10(+) or CD10(−) Bcl-6 (+) MUM1(−) were subclassified as germinal center B-cell type, whereas DLBCL cases with the other expression patterns were subclassified as nongerminal center B-cell type. Regardless of Bcl-6 expression, CD10(−) cases with MUM1(+) were subclassified as nongerminal center B-cell type. Thus, primary breast DLBCL in our cases was subclassified as the nongerminal center B-cell group. Hans *et al* reported that the tissue microarrays for immunohistochemistry divided 152 DLBCL into germinal center B-cell type (64 patients; 42%) and nongerminal center B-cell type (88 patients; 58%) and that the overall survival rates at 5 years for germinal center B-cell type and nongerminal center B-cell type cases were 76 and 34%, respectively. Nongerminal center B-cell type is one of the major predictors of poor DLBCL prognosis. They also mentioned that this classification recapitulated the gene expression results by cDNA microarrays in 71% of germinal center B-cell-like DLBCL and 88% of nongerminal center B-cell-like DLBCL cases, and that it mirrored the predicted for survival in a similar manner. Primary breast DLBCL is a major predictor of poor prognosis.




Hans *et al*[21] also reported that 43% patients in nongerminal center B-cell type expressed Bcl-2 and Bcl-2(+) cases showed significantly worse prognosis than Bcl-2 (−) patients in nongerminal center B-cell type (50 patients; 57%). Two-thirds of the cases in our study were positive for Bcl-2, which is an additional factor for poor prognosis. In addition, the Nordic group reported that 76% of DLBCL expressed CD40 and CD40(+) DLBCL showed better prognosis than CD40(−) DLBCL.[28] In our series, CD40 was positive in only 27% of cases. CD5 expression is known to be a disadvantage for chemotherapy[28,29] and two cases expressed CD5. Immunohistochemical phenotypes of primary breast DLBCL, therefore, can predict their poor prognosis.

Second, we found a very high MIB-1 index of primary breast DLBCL, ranging from 60 to 95%. MIB-1 index has a relationship to prognosis in B-cell lymphomas.[30–32] The MIB-1 index in the other extranodal sites, those of DLBCL with MALT-type lymphoma in the stomach and of DLBCL in the CNS were reported to be 51 and 50%, respectively.[33,34] These clearly indicate an exceptionally high proliferative activity in primary breast DLBCL that may account for the high recurrence rate in stage IE disease.

Although the IgH-V gene showed somatic hypermutations in seven cases ranging from 1 to 10% indicating that the primary breast DLBCL was derived from the germinal center B-cell or the post

**Figure 2** DLBCL with MALT-type lymphoma (case 1). (**a**) Hematoxylin–Eosin (HE) stain shows large-size lymphoma cells on the left side and small- to medium-size cells on the right side. Lymphoepithelial lesion is found in the upper right-hand corner. (**b**) HE stain shows lymphoepithelial lesion by small cell. (**c**) AE1/3 immunostain shows destroyed epithelium (lymphoepithelial lesion).

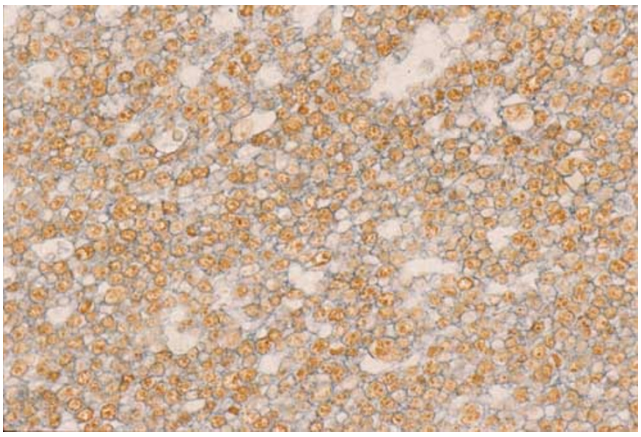


**Figure 3** A MIB-1/CD20-double immunostaining of the paraffin-embedded section (case 8). MIB-1 stains the nuclei, while CD20 stains the cytoplasm and cell membrane. MIB-1/CD20 doubly labelled cells were counted. The mean percentages under three high-power fields was evaluated as the MIB-1 index. Totally, 95.2% of MIB-1 index is found in this slide.

germinal center B-cell,[27,35,36] ongoing mutations were absent in all examined five cases. Germinal center B-cell type DLBCL by cDNA microarray demonstrated the presence of ongoing mutations, but in activated B-cell types DLBCL showed no evidence of ongoing mutations.[37] This also indicated primary breast DLBCL did not belong to the germinal center B-cell type DLBCL.

The frequency of mutations ranged from 1 to 10% for primary breast DLBCL and was similar to those of primary gastrointestinal DLBCL ranging 1.39–15.65%.[38] While the mutation frequency in DLBCL with MALT-type lymphomas (case 1) or with lymphoepithelial lesion (cases 3, 4 and 6) were over 4%, which of DLBCL without MALT-type lymphomas or lymphoepithelial lesion (cases 9 and 13) was less than 3%. This suggests that DLBCL transformed from MALT-type lymphoma and *de novo* DLBCL might demonstrate different values in somatic mutation frequency.

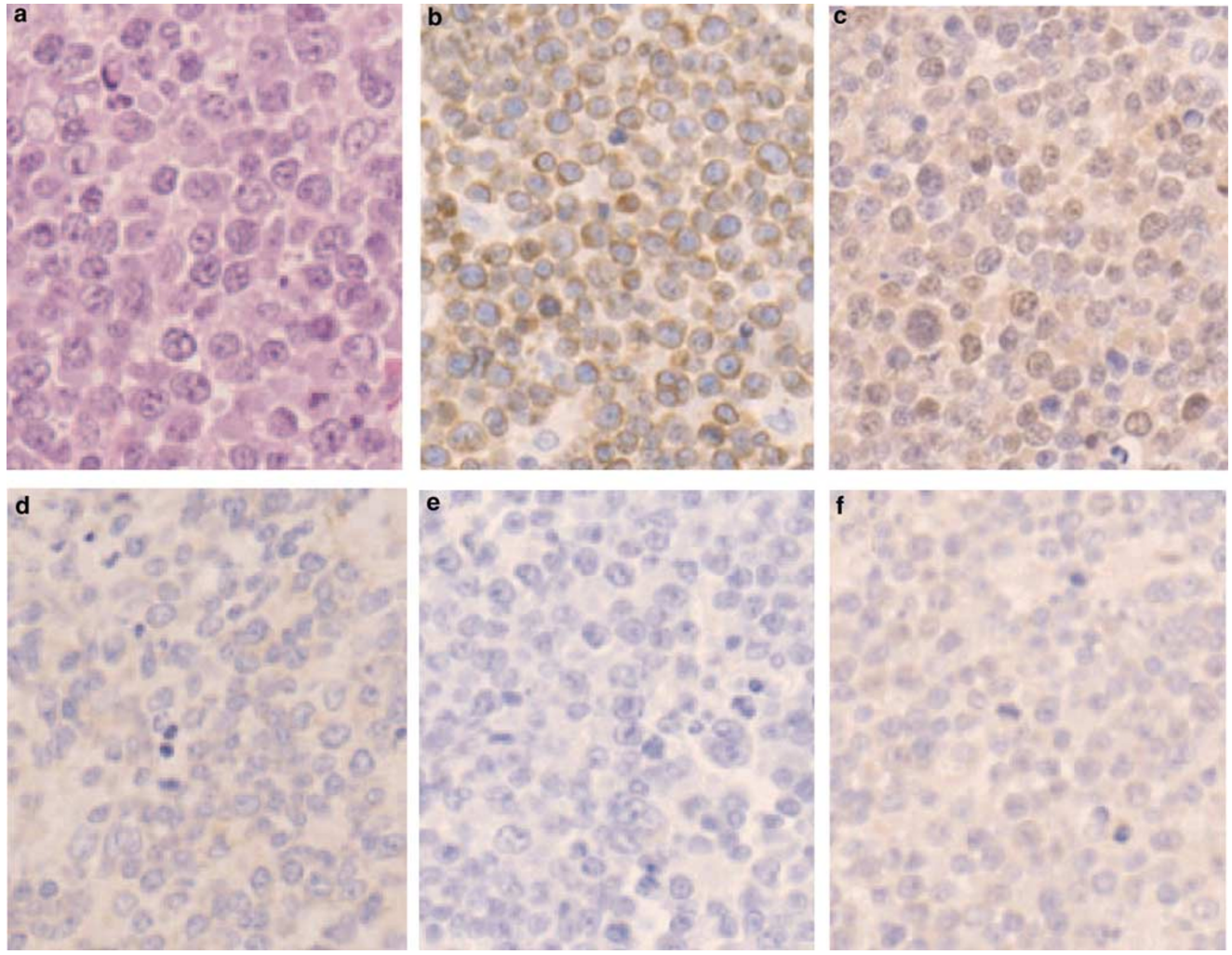



**Figure 4** An immunohistochemical study of primary mammary DLBCL (case 8). (**a**) HE stain of case 8. Bcl-2 (**b**), IRF4/MUM1 (**c**) are positive, CD10 (**d**), CD40 (**e**) and Bcl-6 (**f**) are negative.

**Table 4** Somatic mutations and ongoing mutations found in the immunoglobulin heavy chain gene variable region

| *No.* | *Somatic mutation frequency* | *Closest germ line* | *Additional substitution* | *Ongoing mutation* |
|---|---|---|---|---|
| | *Mutation/total (%)* | | | |
| 1 | 12/162 (7.41) | VH 4-34 | — | NT |
| 3 | 21/204 (10.29) | VH 3- 7 | 1 | No |
| 4 | 11/204 (5.39) | VH 3-23 | 1 | No |
| 5 | 10/201 (4.98) | VH 4-34 | 0 | No |
| 6 | 10/171 (5.85) | VH 4-34 | — | NT |
| 9 | 5/201 (2.49) | VH 4-34 | 0 | No |
| 13 | 3/210 (1.43) | VH 3-15 | 0 | No |

NT: not tested.

The usage of the VH family of primary breast DLBCL was different from other extranodal lymphomas. In the current study, the usage of VH family was VH4 in four and all of them were VH4-34. The remaining three cases used VH3 family. Lossos *et al*[39] reported that 53 cases of nodal and extranodal DLBCL used VH3 most often, followed by VH4, VH1 VH2 H5 and VH7. VH4-34 was used in only five cases. In gastric DLBCL, five of six cases used VH3 family.[38] The high frequency of VH4-34 in primary breast DLBCL might be a feature of this disease.

Recent studies, especially in gastric lymphoma, suggests that *de novo* DLBCL and DLBCL transformed from MALT-type lymphoma is another category in pathogenesis and prognosis,[40,41] but the significant difference in primary breast DLBCL is not clear yet. In our study, DLBCL with or without MALT-type lymphoma showed same nongerminal center B-cell phenotype. Current study, analyzed DLBCL with MALT-type lymphoma is only two cases. More large size studies about germinal center B-cell and nongerminal center B-cell phenotype in primary breast and other extranodal DLBCL with and without MALT type are expected.

In conclusion, primary breast DLBCL with and without MALT-type lymphoma shows nongerminal center B-cell type and high proliferative activity. These dates might be associated with a worse prognosis in primary breast DLBCL.

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